Home Office

NON-TECHNICAL SUMMARY

Generation of antibodies for the study of plant polysaccharides

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man, animals or plants

Key words

plant biology, polysaccharides, antibodies

Animal types	Life stages
Rats	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

We aim to produce antibodies to polysaccharides found in plants. These antibodies will enable us to study the roles of polysaccharides in plant and crop growth.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Plants contain many polysaccharides - which are large and branched polymers of sugars. Antibodies are reagents that are produced by antibody-secreting cells obtained from an animal immune system. Antibodies are important tools to study the roles of these polysaccharides in relation to plant growth and also to develop strategies to improve crops.

What outputs do you think you will see at the end of this project?

Outputs will include antibodies directed to plant polysaccharides released from plant roots. The antibodies will bind specifically to the root polysaccharides and they are needed to track and study the polysaccharides, using fluorescent tags, in roots and during their release into soils. Antibody-based approaches are the most specific and sensitive method to study polysaccharides in cells. There will be publications describing the characteristics of the antibodies and their use to study root-released polysaccharides and their impacts on plant growth and soils. Benefits from this work will include improved cereal crops that will be better able to withstand drought. Once generated the antibodies will additionally be made available to research groups around the world to support plant science research.

Who or what will benefit from these outputs, and how?

Prior work involving plant biochemical and physiological analyses has established our current understanding of the target polysaccharides and whether there is a need for novel specific antibodies. These analyses and decisions will be ongoing during the project. Once isolated, the antibodies will be used in our own research programmes in our laboratory and will immediately extend our understanding of plant growth and the roles of root-released polysaccharides in plant-soil interactions. This work will also benefit the wider academic community upon publication. In the longer term (>5 years), the work will enable crop scientists/breeders to select variants of cereals such as wheat with increased abundance of root-released polysaccharides that will confer growth benefits. Additionally, once details of the antibodies are published they will be made available to all researchers to enable their use to confer benefits in wider research communities.

How will you look to maximise the outputs of this work?

Work with the antibodies will be published as promptly as possible and the antibodies themselves will be made widely available through a dedicated website (www.plantprobes.net) so that academic and commercial research groups around the world can obtain them to support their own research programmes. The antibodies will also be used in collaborative research programmes studying root polysaccharides with barley and wheat geneticists.

Species and numbers of animals expected to be used

- Rats: 20

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Adult rats have been demonstrated to be highly effective for the generation of monoclonal antibodies.

Typically, what will be done to an animal used in your project?

After initial immunisation (primary and secondary), further secondary immunisations may be given to animals. A blood sample will be taken 10 days after the first secondary immunization and if the antibody titre is low (<1,000), another booster immunisation will be given and blood taken again after 10 days. Booster immunisations will be 3-4 weeks apart. This is continued until the titre is suitable enough to lead to a pre-fusion immunisation at some point before or at 6 months from the start. See work plan and decision points in a protocol flow chart in Appendix 1.

What are the expected impacts and/or adverse effects for the animals during your project?

All rats will be in the mild severity category. No adverse impacts or effects are anticipated as the procedures are mild.

In our experience c. 20% of animals produce sufficient antibody titre after primary and secondary immunisation. These animals do not require any further immunisation until the pre-fusion immunisation. The remaining animals do require further immunisation but these injections are always 3-4 weeks apart. In the region of 10% animals fail to produce suitable titre after initial and booster immunisations and are killed by a schedule one method without a pre-fusion injection.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

All rats will be in the mild severity category.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The animal immune system is the most effective way known to isolate cell lines secreting antibodies of appropriate specificities. Non-animal alternatives that continue to be considered through our research are the potential availability of relevant antibodies with appropriate specificities from other laboratories or commercial antibody suppliers, carbohydrate-binding modules from microbial proteins that have some carbohydrate recognition capabilities and also the developing research field of protein-scaffolds, such as affimers/adhirons that have been implemented in synthetic recombinant libraries for screening purposes. To-date these approaches have limited outputs in terms of probes for the target carbohydrate antigens that are the focus of our research interest and currently it is only through using animal immune systems that we can isolate useful tools with appropriate high affinities and high specificities that can drive our research forward.

Which non-animal alternatives did you consider for use in this project?

As mentioned above, non-animal alternatives that are considered through our research are the availability of antibodies with appropriate specificities from other laboratories or commercial antibody suppliers, carbohydrate-binding modules from microbial proteins that have some carbohydrate recognition capabilities and also the developing research field of protein-scaffolds, such as affimers/adhirons that have been implemented in synthetic recombinant libraries for screening purposes.

Why were they not suitable?

No known available antibodies with the required specificity for our target polysaccharides have been generated by other laboratories. Similarly, the microbial carbohydrate-binding modules do not have the range of specificities required to cover our target antigens and in general this set of proteins do not have the desired high affinity and specificity. Research is being carried out to screen for polysaccharide-binding proteins from synthetic recombinant protein scaffold affimer libraries but to date no high affinity, high specificity carbohydrate-directed proteins have been isolated.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

A maximum of 20 animals will be used across 5 years but probably fewer. For the procedure for each target polysaccharide only two animals will be used and this will allow for the use of up to 10 antibody targets across the project if required. Our plant biochemical and physiological research on root polysaccharides will lead to an understanding of their abundance and diversity in several cereal species. Antibodies are the best tools to track and study these polysaccharides in root cells and soils. As our studies develop on root-released polysaccharides we will determine the need for specific antibodies and the research benefits they could provide and only start immunisation procedures if there is a clear requirement for high affinity polysaccharide-specific probes. 20 rats is a maximum and this will allow for use of further animals for immunisation with a specific target polysaccharide if there is a poor immune response. During the last five years during our current licence we have isolated in the region of 15 new useful/effective antibodies (several antibodies can be isolated from the same animal) and some of which are still being characterised.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

The use of animals will be kept to a minimum for the production of cell lines secreting antibodies. Once appropriate cell lines are isolated they will be available indefinitely and produce antibody products for use around the world.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Before any immunisation protocol is started preliminary work on the target polysaccharides will assess the need for antibodies to the target polysaccharide and determine when and how high-affinity antibodies will provide benefits for the continuance of research on the polysaccharides. Alongside this all alternative reagents and approaches will be considered.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Rats are selected for monoclonal antibody generation through hybridoma technology as they provide an efficient system and have proven to be highly effective in the generation of specific antibodies to polysaccharides. Animal suffering will be minimised by keeping animal use to a minimum and all procedures will be carried out by the same trained staff to minimize disruption. A refinement for this project will be the replacement of the previous use of Freund's adjuvants with one of the new adjuvants, such as Titermax, that have been introduced since I have been working in the field. An additional refinement that has the potential to reduce the need for animals during the running of this project include the screening of synthetic recombinant protein scaffold affimer libraries for high affinity proteins that bind specifically to our targets and could be used in our research in place of antibodies.

Why can't you use animals that are less sentient?

The mammalian immune system is the most effective for isolating antibodies and rats are an excellent species for this. Living animals are needed to allow the immune system to respond to target antigens.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Animals will be kept for a minimum period (no more than six months). All interventions and monitoring will be by trained specialist animal care staff.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

Laboratory Animal Science Association (LASA, https://www.lasa.co.uk/) guidelines will be followed by the trained animal care staff.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

Through updates on the 'National Centre for the Replacement, Refinement and Reduction of animals in Research' website (https://nc3rs.org.uk/) and this will include specifically items outlining recommendations for the replacements for animal-derived antibodies and promising new strategies explored.